

through at least one microchannel of the microfluidic device. This flowing can be done electrokinetically, by use of positive or negative pressures, by both electrokinetics and positive or negative pressure, by gravity, by capillary action, through displacement of fluid by expanding membranes, or by centripetal force. Flowing can involve either simultaneous or sequential transport of the molecule(s) through one or more microchannels. Alternatively, the methods of the invention can entail flowing a first molecule through one microchannel and one or more other molecule(s) through a second microchannel, e.g., where the various microchannels intersect with each other.

**[0013]** In the methods in accordance with the invention, heating comprises elevating the temperature of the molecule (s) for a selected period of time. This period of time can range, e.g., from about 0.1 second through to about 1.0 minute or more, from about 0.1 second to about 10 seconds or more, or from about 0.1 second to about 1.0 second or more, including all time periods in between. Optionally, heating can involve raising the temperature of the molecule(s) at a selected point in time after contacting a first molecule by a second molecule. This selected point in time can be from, e.g., about 0.1 second to about 1.0 minute or more, from about 0.1 second to about 10 seconds or more, or from about 0.1 second to about 1.0 second or more (including all time periods in between) after flowing the first molecule into the microchannel. Furthermore, temperature control in the methods can entail setting the temperature of the molecule(s) to a selected temperature that can be from, e.g., about 10° C. to about 100° C. or more, from about 10° C. to about 90° C. or more, or from about 10° C. to about 60° C. or more (including all temperatures in between).

**[0014]** In other methods in accordance with the invention, heating comprises elevating the temperature of the molecule (s) by continuously increasing the temperature of the molecule(s). For example, the temperature of the molecule(s) can be continuously increased at a rate in the range of 0.1° C./second to 1° C./second. Alternatively, the temperature of the molecule(s) can be continuously increased at a slower rate, such as a rate in the range of 0.01° C./second to 0.1° C./second, or at a faster rate, such as a rate in the range of 1° C./second to 10° C./second.

**[0015]** Heating the molecules optionally comprises elevating the temperature of the molecule(s) in the microchannel by either joule heating, non-joule heating, or both joule heating and non-joule heating. In one embodiment, joule heating is performed by flowing a selectable electric current through the microchannel, thereby elevating the temperature. Joule heating can occur over the entire length of the microchannel or over a selected portion of the microchannel. Joule heating can be applied to selected portions of microchannels by flowing a selectable electric current through a first section and a second section of a microchannel wherein the first section comprises a first cross-section and the second section comprises a second cross-section. Furthermore, the first cross-section is of a greater size than the second cross-section, which causes the second cross-section to have a higher electrical resistance than the first cross-section, and therefore a higher temperature than the first cross-section when the selectable electric current is applied. The level of joule heating can be controlled by changing the selectable current, the electrical resistance, or both the current and the resistance. The selectable current used for joule heating can include direct current, alternating

current or a combination of direct current and alternating current. See, e.g., U.S. Pat. No. 5,965,410.

**[0016]** Optionally the heating used in the methods of the invention includes non-joule heating, e.g., through application of an internal or an external heat source. In one embodiment, the internal or external heat source includes a thermal heating block. Just as for joule heating, non-joule heating optionally occurs over the entire length of the microchannel or over a selected portion of the microchannel. For example, one or more regions of the microchannel can be proximal to one or more heating element.

**[0017]** Heating methods in accordance with the invention also encompass the application of a temperature gradient along the length of a portion of a microchannel. The temperature at one end of the length of the microchannel is controlled to a first selected temperature, and the temperature at the other end of the length is controlled to a second selected temperature, thus creating a continuous temperature gradient spanning the temperature range between the first and second selected temperatures. Once a steady state flow of fluid through the portion of the microchannel is established, a temperature gradient will be established within that fluid. When Joule heating is used, a temperature gradient can be established along the length of a microchannel by fabricating the channel so that it continuously and monotonically changes in cross-sectional area along its length, and then applying a single electric current through that length. One method of establishing a temperature gradient along the length of a microchannel when non-joule heating is employed is to place a thermal block in contact with the microchannel, and to establish a temperature gradient across the block in the direction corresponding to the length direction of the microchannel using heating or cooling elements.

**[0018]** In another aspect of the invention, the methods of detecting a property of the molecule(s) involved comprises detecting a level of fluorescence or emitted light from the molecule(s) that varies as a function of relative amounts of binding. In one configuration, the detecting of fluorescence involves a first molecule and a second molecule, wherein the first molecule is a fluorescence indicator dye or a fluorescence indicator molecule and the second molecule is the target molecule to be assayed. In one embodiment, the fluorescence indicator dye or fluorescence indicator molecule binds or associates with the second molecule by binding to hydrophobic or hydrophilic residues on the second molecule. The methods of detecting optionally further comprise exciting the fluorescence indicator dye or fluorescence indicator molecule to create an excited fluorescence indicator dye or excited fluorescence indicator molecule and discerning and measuring an emission or quenching event of the excited fluorescence indicator dye or fluorescence indicator molecule.

**[0019]** In an illustrative embodiment of a method involving detection of fluorescence involves a molecule that comprises a protein or a polypeptide. In this embodiment, the method of detecting further entails exciting amino acid residues such as tryptophan in the protein or polypeptide, thereby creating excited tryptophan residues. Discerning and measuring an emission or quenching event of the excited tryptophan residues is used to detect a property of the molecule(s) being assayed.

**[0020]** In addition, or separate from, fluorescence or emitted light detection, detecting a property of the molecule(s) being assayed may optionally comprise the use of, e.g., fluorescence spectroscopy involving, e.g., fluorescence polariza-